Seeing In the Dark

Measuring thermal events in cells and behavior

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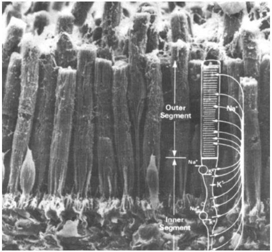
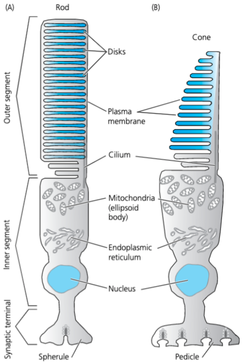
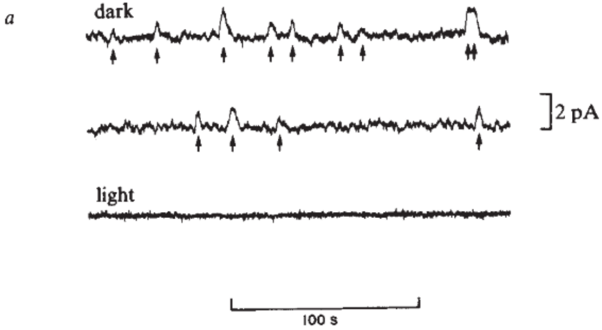
Physics/Neuro 141

Week 3



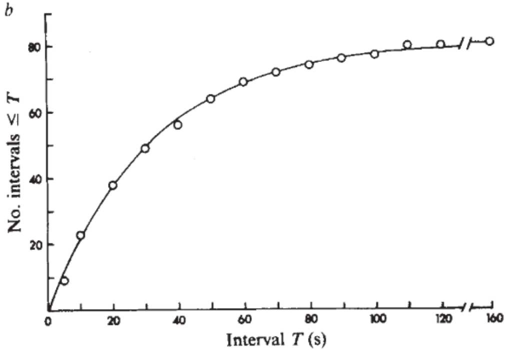


Vertebrate rods and cones. Principal structural features of vertebrate photoreceptors. (A) Rod. The outer segment is composed of disks detached from external plasma membrane. (B) Cone. The outer segment has membrane infoldings or lamellae instead of disks



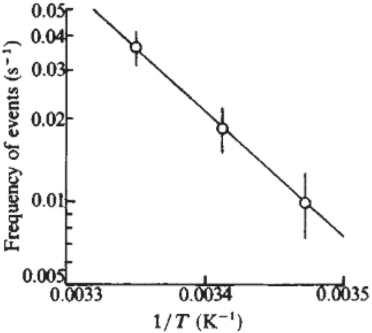
Sample records of rod outer segment current in darkness (two upper traces) and bright light (bottom trace)

Poisson interval distribution



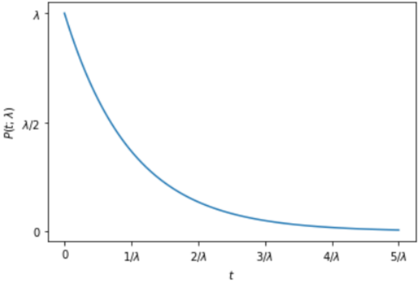
Cumulative distribution of intervals between successive events.
Continuous curve corresponds to: $n = N\left(1 - \exp(-T/\tau)\right)$

Arrhenius Law for Thermal Dependence of Re-acttion Rate



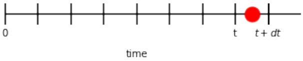
Arrhenius plot of frequency

Poisson Interval Distribution



Events that occur with probability per unit time λ will have a mean waiting time $\langle t \rangle = \lambda$. The probability of the next event occurring in an interval between t and $t + dt$ will be exponentially distributed: $P(t)dt = \lambda e^{-\lambda t} dt$. Shorter waiting times are more likely.

Poisson interval distribution



- Consider a discrete event that occurs with probability per unit time *lambda*
- Divide the timeline from 0 to t into a large number N intervals, $\Delta t = t/N$.
- The probability of *non-occurrence* in each interval is $1 - \frac{\lambda t}{N}$. Thus, the probability of non-occurrence in all N intervals is $\left(1 - \frac{\lambda t}{N}\right)^N$, hence

$$\lim_{N \to \infty} \left(1 - \frac{\lambda t}{N}\right)^N = e^{-\lambda t}$$

- The probability of *occurrence* in the last interval is λdt

$$\boxed{P(t)dt = \lambda e^{-\lambda t}}$$

- Instead of recording intervals, suppose we count the number of events that occur in a fixed time t'. The probability that an event does *not* occur in the interval t' is one minus the probability that an event does occur:

$$1 - \int_0^{t'} \lambda e^{-\lambda t} dt = e^{-\lambda t'}$$

- The probability that **one** event occurs in the interval t' is equal to the probability that an event occurs between t_0 and $t_0 + dt_0$ times the probability that an event does not occur in the rest of the interval, $t' - t_0$, integrated over all values of t_0:

$$\int_0^{t'} \lambda e^{-\lambda t_0} e^{-\lambda(t' - t_0} dt_0 = \lambda t' e^{-\lambda t'}$$

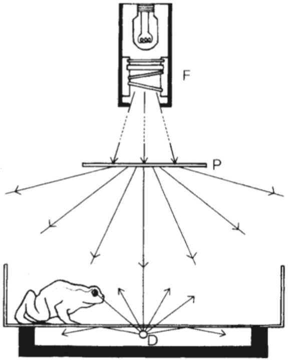
- The probability that **two** events occur in the interval t' is equal to the probability that an event occurs between t_0 and $t_0 + dt_0$ times the probability that one event occurs in the rest of the interval, $t' - t_0$, integrated over all values of t_0:

$$\int_0^{t'} \lambda e^{-\lambda t_0} \lambda(t' - t_0) e^{-\lambda(t' - t_0} dt_0 = \frac{(\lambda t')^2}{2} e^{-\lambda t'}$$

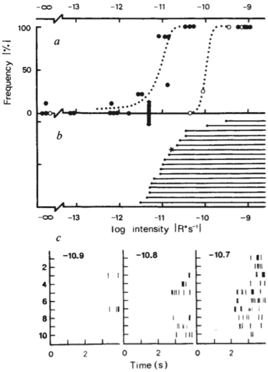
- Proceeding in this fashion, the probability that k events occur in the interval of time t' is:

$$\boxed{P(k; \mu) = \frac{\mu^k}{k!} e^{-\mu}}$$

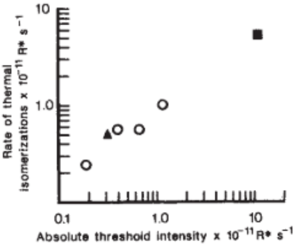
Low-noise at low temperatures



A fully dark-adapted toad in a plastic box was illuminated from above by a green light. Underneath the floor, white ‘worm dummies’ moved over a black background. The occurrence of one or several snaps indicated that the toad had seen a worm.



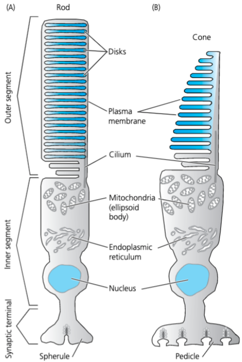
- Behavioral (a) and electrophysiological (b,c) determinations of absolute sensitivity.
- The abscissa indicates intensity in “photoisomerizations per Rhodopsin per second”
- Thermal isomerization rate is shown by the black arrow
- Frequency of seeing for the toad is shown by filled circles
- Frequency of seeing for a human is shown by open circles



Correlation between rates of thermal rhodopsin isomerization and absolute threshold intensities, expressed as rates of isomerization per rhodopsin molecule in the retina of the toad, frog, and man

The optimum length of a rod cell?

Longer rods → more photon absorption

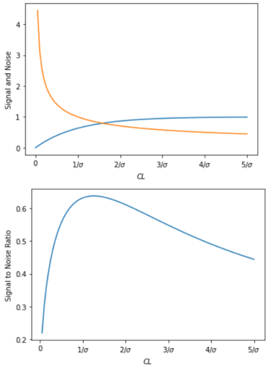


- Consider a rod cell of length L, cross-sectional area A, and concentration of rhodopsin C.
- We can determine the mean number of potential absorption events by multiplying the probability of absorption by a single rhodopsin molecule by the number of rhodopsin molecules in the path of the incident photon.
- Assuming that the photon travels parallel to the axis of the rod cell, this mean number $\mu = \sigma CL$.
- Absorption events are Poisson distributed, and the probability of capturing a photon is equal to the probability of not getting zero captures.
- The probability of a positive result from a Poisson process is:

$$p_{\text{abs}} = 1 - e^{-\mu} \quad (1)$$

$$= 1 - e^{-\sigma CL}. \quad (2)$$

Optimum length



- If a rhodopsin has a probability p_{dark} of being activated by temperature, the mean number of activations will be this probability multiplied by the total number of rhodopsins in the cell:

$$\mu_{\mathrm{dark}} = CLAp_{\mathrm{dark}}. \tag{3}$$

- Since dark events are also Poisson distributed, the standard deviation in the number of dark events $\sigma_{\mathrm{dark}} = \sqrt{\mu_{\mathrm{dark}}}$.
- Assuming that the photon travels parallel to the axis of the rod cell, this mean number $\mu = \sigma CL$.
- The signal-to-noise ratio of this rod cell when a flash of *n* photons is delivered to it is:

$$SNR = \frac{np_{\mathrm{abs}}}{\sqrt{\mu_{\mathrm{dark}}}} \tag{4}$$

$$= n\frac{1 - e^{-\sigma CL}}{\sqrt{CLAp_{\mathrm{dark}}}}. \tag{5}$$

Retinal Noise and Absolute Threshold

Horace Barlow, 1956

- It is shown that the absorption of one quantum can excite a rod in the human retina, but that at least two, and probably many more, excited rods are needed to give a sensation of light.
- It is suggested that noise in the optic pathway limits its sensitivity, and this idea is subjected to an experimental test.
- The hypothesis is then formulated quantitatively, and shown to be able to account for the above experiment, and also the disagreement in the literature between those who believe that the absorption of two quanta can cause a sensation, and those who believe that 5 or more are required.
- The formulation of the hypothesis is used to calculate the maximum allowable noise (expressed as a number x of random, independent events confusable with the absorption of a quantum of light) in the optic pathway for the absorption of various fractions of the total number of quanta incident at the cornea.

More than one quantum and more than one rod is needed to see

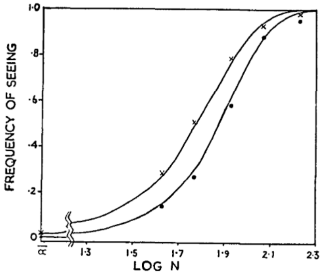
Hecht Shlaer and Pirenne

- 500 rods
- 5-8 photons
- What is the likelihood of double hits?

Denton and Pirenne

- 70,000 rods
- 280 rods
- What is the likelihood of double hits?

Frequency of seeing and possibly seeing, Barlow (1956)



- Subject was looking at flashes of light similar to Hecht et al
- Signaled when he saw a flash for each of 100 presentations at five intensities
- 300 blanks were mixed in (so we can measure false positive rate)
- The fraction of ‘seen’ form the right hand set of dots
- The fraction of ‘seen + possibly seen’ form the left hand set of dots
- The subject never ‘saw’ a blank, but ‘possibly saw’ 1% of the blanks

Rods do not distinguish 'dark' events and 'light' events by themselves

What the rod detects

- N = number of photons that land on the cornea
- n = average number of independent events resulting from a stimulus flash
- x = average number of noise events confusable with the stimulus events
- $a = n + x$ = total number of rod excitations

What the subject sees

- c = the number of events which must be equalled or exceeded to get a response
- $P_{see}(a) =$ = probability of c or more events occurring if the average number is a

$$P_{see}(a) = \sum_{y=c}^{\infty} \frac{a^y e^{-a}}{y!}$$

- $a = n + x$ = total number of rod excitations

The probability of seeing curve depends on threshold and noise

The slope of the probability of seeing curve

- We measure the slope of the probability of seeing curve with respect to a logarithmic scale of light intensity: $\frac{dP_{see}}{d(\log N)}$
- Of the photons that hit the cornea (N), a fixed fraction are absorbed by the retina ($n/N = const$). Thus,

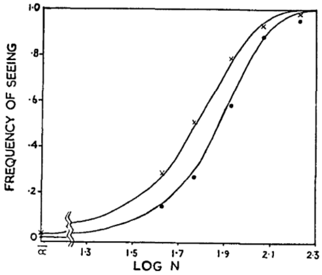
$$d \log N = d \log n$$

- $$\frac{dP_{see}}{d(\log N)} = \frac{dP_{see}}{d(\log n)} = \frac{dP_{see}}{da} \times \frac{da}{d \log n} = \frac{e^{-a} a^{c-1}}{(c-1)!} \times n$$

- Use Stirling's formula $\boxed{n! \sim \sqrt{2\pi n} \left(\frac{n}{e}\right)^n}$. At threshold, $a = n + x = c$:

$$\frac{dP_{see}}{d(\log N)} \times (2\pi)^{1/2} = \frac{c - x}{c^{1/2}}$$

Fitting to *two* probability of seeing curves



- $\frac{n}{N} \approx 0.14$
- $x \approx 8.9$
- For the possible threshold: $c \approx 17$
- For the seen threshold: $c \approx 19$

The reason our threshold of seeing is several photons is *because* of dark noise.